Catalytic Center Assembly of HPPK as Revealed by the Crystal Structure of a Ternary Complex at 1.25 Å Resolution

J. Blaszczyk and X. Ji (NCI), G. Shi and H. Yan (Michigan State U.)

Abstract No. blas9533
Beamline(s): **X9B**

Introduction: Folates are essential for life. Unlike mammals, most microorganisms must synthesize folates *de novo*. 6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) catalyzes pyrophosphoryl transfer from ATP to 6-hydroxymethyl-7,8-dihydropterin (HP), the first reaction in folate pathway, and therefore, is an ideal target for developing novel antimicrobial agents. HPPK from *Escherichia coli* contains 158 amino acid residues and is thermostable, and thus, is a convenient model system for mechanistic studies. Crystal structures have been reported for HPPK without bound ligand (PDB entry 1HKA, 1998), containing an HP analog (1CBK, 1999), and complexed with an HP analog, two Mg^{2+} ions, and ATP (1DY3, 2000).

Methods and Materials: Single crystal X-ray diffraction.

Results: We present the 1.25 Å crystal structure of HPPK in complex with HP, two Mg^{2+} ions, and AMPCPP (an ATP analog that inhibits the enzymatic reaction), which shows that the enzyme seals the active center where the reaction occurs. The comparison with unligated HPPK reveals dramatic conformational changes of three flexible loops and many side chains. The coordination of Mg^{2+} ions has been defined and the roles of 26 residues have been derived.

Conclusions: HPPK•HP•MgAMPCPP mimics most closely the natural ternary complex of HPPK and provides details of protein-substrate interactions. The coordination of the two Mg^{2+} ions aids in creating the proper geometry for the one-step reaction of pyrophosphoryl transfer, for which we suggest an in-line single displacement mechanism with some associative character in the transition state. The rigidity of adenine-binding pocket and hydrogen bonds are responsible for adenosine specificity. The nonconserved yet substrate-interacting residues may be responsible for the species-dependent properties of an isozyme.

Acknowledgments: This work was supported in part by NIH grant GM51901 to HY. We thank Dr. Zbigniew Dauter for his kind assistance and suggestions during data collection and processing at synchrotron beamline X9B in Brookhaven National Laboratory.